H (0.1 U/μl) in a buffer containing 20 mM Hepes, pH 7.9, 10 mM $MgCl_2$, 50 mM KCl, 1 mM DTE, and the DNA oligonucleotide 5'-CTCTAGAGTCGACCTGCAGCCCAAG-3' (0.1 μg/μl). After incubation of the reaction mixture for 1.5 hours at 37°C, the DNA oligonucleotide was digested with DNase I. The DNA was finally extracted and precipitated as described above and resuspended in water.
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Hypertriglyceridemia as a Result of Human Apo *CIII* Gene Expression in Transgenic Mice

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Primary and secondary hypertriglyceridemia is common in the general population, but the biochemical basis for this disease is largely unknown. With the use of transgenic technology, two lines of mice were created that express the human apolipoprotein *CIII* gene. One of these mouse lines with 100 copies of the gene was found to express large amounts of the protein and to be severely hypertriglyceridemic. The other mouse line with one to two copies of the gene expressed low amounts of the protein, but nevertheless manifested mild hypertriglyceridemia. Thus, overexpression of apolipoprotein *CIII* can be a primary cause of hypertriglyceridemia in vivo and may provide one possible etiology for this common disorder in humans.

HYPERTRIGLYCERIDEMIA (HTG) IS common in the general population, particularly in people with premature coronary heart disease (*1, 2*). HTG can occur on a primary genetic basis or be associated with conditions such as diabetes mellitus, obesity, pancreatitis, chronic renal failure, alcoholism, stress, or infection (*3*). Plasma triglycerides are carried principally in chylomicrons and very low density lipoprotein (VLDL). Metabolic turnover studies indicate that increased production or decreased catabolism of these lipoproteins, or both, can occur in patients with HTG (*4, 5*). In the majority of these patients, the cause of altered synthesis or breakdown of the triglyceride-rich lipoproteins is not known. In familial hyperchylomicronemia (Type I hyperlipoproteinemia), a functional defect exists in the lipoprotein lipase enzyme, which hydrolyzes the triglycerides in chylomicrons and VLDL. This is due to either defects in the lipoprotein lipase gene or defects in the gene that codes for its cofactor apolipoprotein (apo) CII (*6–9*). The frequency of these individuals in the general population is less than one in a million.

In our studies of the cis-acting DNA sequences required for tissue-specific expression of the apolipoprotein genes, we introduced the intact human apo *CIII* gene with flanking sequences into mice. The apo CIII protein is a major constituent of chylomicrons and VLDL. Apo *CIII* is 3.1 kb in length and maps within an apolipoprotein gene cluster on human chromosome 11q23, between the apo *A-I* and apo *A-IV* genes. Apo *CIII* contains four exons and encodes a mature protein of 79 amino acids (*10–13*). Two different lines of transgenic mice that expressed human apo *CIII* were established. In a line containing approximately 100 copies of the human gene, the mice were severely hypertriglyceridemic. In a second line, animals with one to two copies of the human gene manifested mild HTG. These experiments revealed that in an in vivo animal model, overexpression of apo *CIII* can lead to HTG, and suggest one possible etiology for this disorder in humans.

A recombinant bacteriophage clone containing the human apo *CIII* λ apo *A-I-8* (*10, 13*) was subjected to restriction endonuclease digestion and yielded a 6.7-kb DNA fragment between the Kpn I site 2.5 kb 5′ to apo *CIII* and the Xba I site 1.1 kb 3′ to the gene. This DNA fragment was gel-purified and used for microinjection. Transgenic mice were created as previously reported (*14*). Approximately 400 copies of human apo *CIII* were injected into the male pronucleus of fertilized eggs from superovulated (C57BL/6J × CBA/J) F1 females that had been mated to males of the same genetic background. Injected eggs were surgically transferred to oviducts of surrogate females. Of 26 mice born, 3 had integrated human apo *CIII*. Two mice were bred to (C57BL/6J × CBA/J) F1 mice, and two transgenic lines were established (2721 and 2674). The inheritance pattern of the transgene in both lines was compatible with a single autosomal integration site.

We determined the copy numbers of human apo *CIII* in these two transgenic lines with quantitative Southern (DNA) blotting

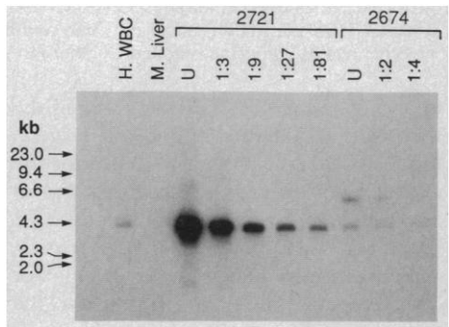

Fig. 1. Quantitative Southern blotting analysis of human apo *CIII* transgenic mouse lines 2721 and 2674. DNA was extracted from tail tissue of two lines of transgenic mice (Tg) expressing apo *CIII*, digested with Pst I, subjected to electrophoresis in a 0.7% agarose gel, transferred to a nylon membrane (Oncor), and hybridized with a 517-bp human apo *CIII* cDNA probe derived from pCIII-607 (*15*). Tg line 2721 DNA was loaded in serial dilutions of 10 μg [undiluted (U)], 3.33 μg (1:3), 1.11 μg (1:9), 0.37 μg (1:27), and 0.12 μg (1:81). Similarly, DNA from a progeny of the founder mouse Tg-2674 was loaded in dilutions of 10 μg (U), 5 μg (1:2), and 2.5 μg (1:4). The cDNA probe hybridized to a 4.2-kb band representing a Pst I fragment beginning at the Pst I site 200 bp 5′ to the start of transcription of the human apo *CIII* and terminating at the Pst I site located approximately 900 bp 3′ to the gene. A copy number of 100 to 110 for Tg line 2721 and 1.8 for Tg line 2674 was obtained by a densitometric scan of the 4.2-kb band, after normalization for the human white blood cell (H. WBC) signal. The additional band at 6 kb in the Tg line 2674 blot probably results from the loss of one of the apo *CIII* flanking Pst I sites in an end copy of the human genomic DNA insert. Control, mouse liver DNA (M. Liver).

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analysis (Fig. 1). Genomic DNA was digested with Pst I, and the resultant blots were probed with a human apo *CIII* cDNA probe, pCIII 607 (*15*). Human apo *CIII* is contained between two flanking Pst I sites that are 4.2 kb apart, and both transgenic lines have the corresponding band, which is not seen in genomic DNA from control mouse liver. Comparison of the intensity of the signal from the 4.2-kb human apo *CIII* fragment in transgenic lines to that in human DNA from white blood cells (WBC) (two copies) indicated that transgenic line 2721 has approximately 100 copies and line 2674 approximately 1 to 2 copies of human apo *CIII*.

Expression of human apo *CIII* mRNA was verified by a solution hybridization ribonuclease (RNase) protection assay with a riboprobe containing a cDNA sequence from the human apo *CIII* 3′ untranslated region (Fig. 2). When labeled riboprobe (220 bases in length) was incubated with RNA from a human hepatoma cell line (HepG2) that synthesizes apo CIII, we observed a 147-base protected fragment, as predicted. With RNA from liver and intestine of both transgenic lines, we also observed a protected 147-base fragment, indicating that human apo *CIII* was being expressed. No protected band was observed when brain RNA from the transgenic lines was used in the RNase protection assay. In addition, no protected band was detected in liver, intestine, or brain RNA from a control animal. In both transgenic lines, expression in liver exceeded that in the intestine. Line 2721 with 100 copies of human apo *CIII* has considerably more apo *CIII* mRNA than line 2674 with only 1 to 2 copies of the human apo *CIII*. Thus, we referred to line 2721 as a high expressor and line 2674 as a low expressor.

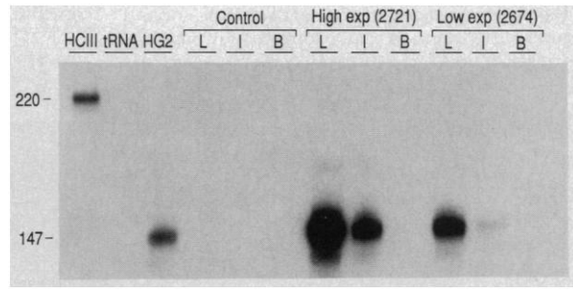

Fig. 2. RNase protection of human apo *CIII* mRNA. Human apo *CIII* mRNA-specific riboprobe (50 μg) was hybridized to 5 μg each of yeast tRNA (tRNA), Hep G2 cell RNA (HG2), or RNA isolated from liver (L), small intestine (I), and brain (B) of either control mice or high-expressor [High exp (2721)] or low-expressor [Low exp (2674)] transgenic mice at 63°C in 25 μl of 40 mM Hepes, pH 6.7, 400 mM NaCl, 1 mM EDTA, and 80% formamide for 3.5 hours. The samples were treated with 5 units of RNase A and 3.6 units of RNase T_1 in 300 μl of 300 mM NaCl, 10 mM tris-HCl, pH 7.4, and 5 mM EDTA at 34°C for 45 min. Addition of 10 μl of 20% SDS and 100 μg of proteinase K was followed by incubation at 37°C for 15 min and ethanol precipitation. RNase-protected fragments were subjected to electrophoresis in an 8% polyacrylamide, 8 M urea gel, and autoradiographed. The sizes of gel bands were determined by direct comparison to ^{32}P-labeled, Msp I–cut pBR322 restriction fragments. The human apo *CIII* riboprobe was prepared from a human apo *CIII* cDNA clone pCIII-607 (*15*), digested with Pst I and Sac I, and the 147-bp fragment was isolated and subcloned into the Pst I–Sac I sites of PGem1 (Promega). The vector was linearized 5′ to the *CIII* cDNA, and SP6 RNA polymerase was used to produce a ^{32}P-labeled riboprobe of 220 nucleotides in length.

We next studied effects of human apo *CIII* expression on plasma apo CIII and triglyceride levels (Fig. 3). Fasting plasma from high-expressor transgenic animals had a milky appearance, similar to plasma from an individual with severe HTG (Type V hyperlipoproteinemia). Plasmas from controls as well as low-expressor transgenic animals were clear. Cloudy plasma is indicative of HTG, and indeed, the plasma specimen from the high-expressor animal had triglycerides of 1488 mg/dl, whereas the control mouse plasma triglycerides were 40 mg/dl. Portions of the same plasma specimens in Fig. 3 were used in an electroimmunoassay with an antibody to human apo CIII that does not cross-react with mouse apo CIII (Fig. 3). When compared to control human plasma, the high-expressor animal had a greatly increased amount of human apo CIII in its plasma, comparable to plasma levels seen in patients with Type V hyperlipoproteinemia. In six G_1 high-expressor animals, the human apo CIII concentration was 54 ± 11 mg/dl and the triglycerides were 959 ± 217 mg/dl; in individual animals, there was a linear relationship of human apo CIII and triglyceride concentrations with a slope of 1.14 (Table 1 and Fig. 4).

These results show that a large amount of apo *CIII* overexpression in vivo can cause HTG. We next turned our attention to the low-expressor animals to see if even a small amount of apo *CIII* overexpression in vivo

Table 1. Effect of human apo *CIII* expression on plasma triglyceride and apolipoprotein concentrations. Fasting triglyceride and human apo CIII concentrations were determined as described in Fig. 4. Ten percent sucrose was administered in drinking water over 12 hours before bleeding without access to food. For fat tolerance test, 400 μl of olive oil was bolus-injected into the stomach after 12-hour fasting under methoxyflurane anesthesia. Approximately 90 μl of blood was collected serially at 0, 30, 60, 120, 180, and 300 min for triglyceride determinations. Mouse apo A-I concentrations were quantitated by densitometric scanning of nonreducing SDS–polyacrylamide gel electrophoresis patterns of plasma proteins. Values are mean ± SEM. *P = 0.1, compared to negative littermates. **$P < 0.05$ comparison among three groups by the Duncan's multiple range test. ***P = 0.01 compared to negative littermates. ****P = 0.0001 compared to heterozygotes. *****P = 0.002 compared to other groups. ND, not determined; TG, triglycerides.

Animals	Fasting			10% sucrose feeding		Bolus fat feeding
	Human apo CIII[a]	TG (mg/dl)	Mouse apo A-I	Human apo CIII (mg/dl)	TG (mg/dl)	TG area under the curve (mg/dl × min)[b]
			High expressor			
G_1 (2m, 4f)[c]	54 ± 11	959 ± 217	59 ± 9*****	ND	ND	
			Low expressor			
G_1						
Negative littermate (7m)		49 ± 6	117 ± 7		99 ± 25	22,224 ± 2,510
Heterozygous (7m)	3.1 ± 0.3	67 ± 8*	108 ± 12	3.4 ± 0.3	141 ± 24	34,935 ± 3,676***
G_2						
Negative littermate (1m, 2f)		58 ± 8**	111 ± 12	ND	ND	
Heterozygous (6m, 1f)	3.0 ± 0.3	173 ± 23**	108 ± 7	ND	ND	
Homozygous (2m, 1f)	8.2 ± 1.0****	395 ± 70**	110 ± 10	ND	ND	

[a]Normotriglyceridemic human subjects (plasma triglyceride concentrations 117 to 156 mg/dl, n = 8) had CIII concentrations of 7.8 ± 0.7 mg/dl in this assay. [b]Area under the curve of triglyceride concentration (mg/dl) over a time span of 300 min. [c]The number of males (m) and females (f) in each group is shown in parentheses.

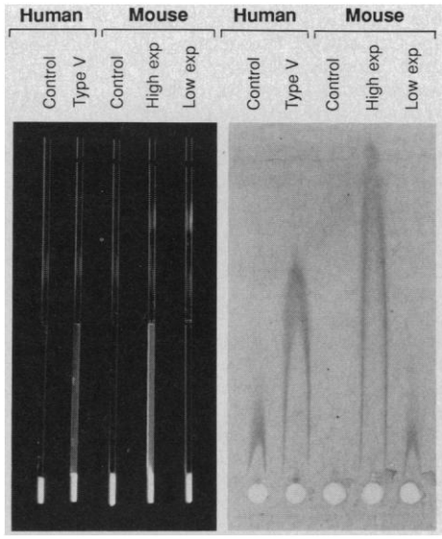

Fig. 3. Appearance of representative plasma specimens (left) and their electroimmunoassay pattern (right). Human and mouse blood was collected after a 12-hour fast, and the plasma was separated by centrifugation at 1875*g* for 10 min. The human blood was collected from an antecubital vein and the mouse blood from the retro-orbital plexus under methoxyflurane anesthesia. Triglyceride levels, measured by enzymatic assay (126012, Boehringer Mannheim), were 123, 1333, 40, 1488, and 50 mg/dl, respectively, in human control and Type V hyperlipoproteinemic specimens and mouse control and transgenic high- and low-expressor specimens. Human apo CIII was measured by electroimmunoassay. Seven microliters of 1:31 (v/v) diluted plasma with 0.125% Tween 20 in 0.9% NaCl was applied to an agarose gel containing 0.8% agarose, 2% dextran T70 (Pharmacia), and 2% goat polyclonal antibody to human apo CIII (Daiichi Pure Chemicals, Tokyo, Japan) in tris–Tricine IV buffer (pH 8.6, Bio-Rad). The agarose gel was run in this tris–Tricine IV buffer at constant voltage of 40 for 13 hours, extensively washed in 0.9% NaCl, stained with 0.1% Coomassie blue R-250 in 30% methanol, 10% acetic acid, and destained with water. Plasma apo CIII levels were determined by comparing an area under the curve of each specimen; measurements were made with an area estimator (170–4097, Bio-Rad) and compared with those of purified apo $CIII_2$ standards (*34*). The interassay coefficient of variation was 10%.

could increase plasma triglyceride concentrations. In one group of low-expressor transgenic animals, the mean human apo CIII concentration was 3.1 ± 0.3 mg/dl (the mean for humans with normal triglyceride concentrations was 7.8 ± 0.7 mg/dl). In these mice, fasting triglycerides were 67 ± 8 mg/dl versus 49 ± 6 mg/dl in controls ($P = 0.1$) (Table 1). These animals were again studied 12 hours after food withdrawal but were offered 10% sucrose during this time period. Sucrose feeding is known to increase endogenous triglyceride and VLDL production (*16*) and constitutes a stress on the triglyceride removal system. In this case, triglycerides in the transgenic animals were 141 ± 24 mg/dl compared to 99 ± 25 mg/dl in the controls (Table 1). The trend to higher triglyceride concentrations in the low-expressor apo *CIII* transgenic animals was not significant; however, results from one of the control animals was outside the range of the other animals. Eliminating the outlier value gave a triglyceride value of 74 ± 12 mg/dl in the controls, which was significantly different from the transgenic animals ($P < 0.03$). Finally, these animals were subjected to a dietary fat challenge. After 12 hours of fasting, a bolus of fat (400 μl of olive oil) was introduced intragastrically, and plasma triglycerides were measured periodically for 5 hours. The area under the plasma triglyceride concentration curve was computed (*17*), and the results for the transgenic animals were significantly greater than for the nontransgenic littermates (Table 1).

Subsequent experiments were performed with a different group of low-expressor animals. G_1 transgenic animals with the highest plasma triglyceride values were mated, producing G_2 animals with 0 ($n = 3$), 1 ($n = 7$), and 2 ($n = 3$) copies of the transgene. These G_2 animals had fasting triglyceride levels of 58 ± 8, 173 ± 23, and 395 ± 70 mg/dl, respectively, with a significant gene dosage effect on plasma triglyceride concentrations. There was also a significant effect of gene dosage on plasma concentration of human apo CIII when we compared heterozygous and homozygous littermates, 3.0 ± 0.3 and 8.2 ± 1.0 mg/dl, respectively (Table 1). The experiments with the G_1 and G_2 animals of the low-expressor line indicate that even low levels of apo *CIII* overexpression can have an effect on plasma triglyceride levels.

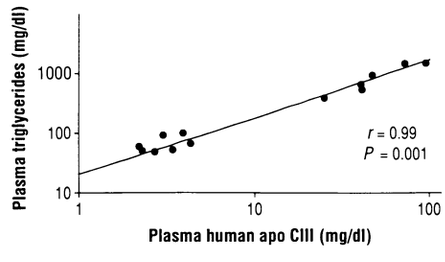

Fig. 4. Correlation between plasma triglyceride (TG) levels and human apo CIII concentration. G1 mice (six high expressors and seven low expressors) (Table 1) were used in this analysis. The regression equation was $\log_{10}TG$ (mg/dl) = 1.36 + 0.93$\log_{10}$human apo CIII (mg/dl). In high expressors, the regression equation was $\log_{10}TG = 1.0 + 1.14\log_{10}CIII$ (SE of slope 0.15, $P = 0.002$), and that in low expressors was $\log_{10}TG = 1.53 + 0.58\log_{10}CIII$ (SE of slope 0.46, $P = 0.26$).

Finally, the plasma lipoprotein profile in control, low-, and high-expressor animals was evaluated by column chromatography (Fig. 5) (*18*). The predominant particles that accumulate in the plasma of the transgenic animals were the size of VLDL. There also appeared to be a diminution of the high-density lipoprotein (HDL) peak in the high-expressor animal, which is compatible with the decrease in mouse apo *A-I* we found in the plasma of the high-expressor animals (Table 1).

The CIII protein is a major constituent of chylomicrons and VLDL (50% of the pro-

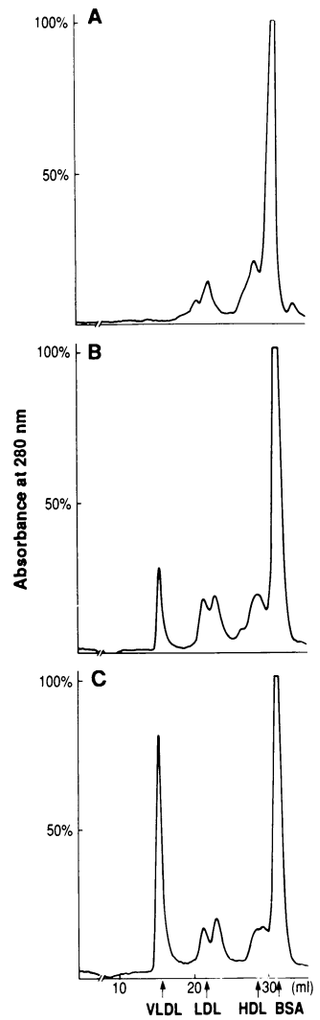

Fig. 5. Gel filtration chromatography of (**A**) control, (**B**) homozygous low-expressor, and (**C**) high-expressor mice. Fasting plasma (10 μl) [triglyceride levels: (A) 50, (B) 536, and (C) 864 mg/dl] was diluted with 240 μl of column buffer (0.15 M NaCl and 1 mM EDTA, pH 7.4), and injected into two Superose 6 HR 10/30 columns (Pharmacia) in sequence (each column is 1 by 30 cm), chromatographed at a rate of 0.35 ml/min for 50 ml (Fast Performance Liquid Chromatography system, Pharmacia_LKB) (*18*). Peak retention volumes of mouse VLDL, human low density lipoprotein (LDL), mouse HDL, and bovine serum albumin (BSA) are indicated by arrows.

tein of VLDL) and plasma apo CIII is elevated in HTG (*19, 20*). It had been thought that the elevation in plasma apo CIII concentration that occurs in HTG was a secondary effect due to a combination of increased production or decreased catabolism of VLDL particles. Using transgenic animals, we have shown that a primary overproduction of apo CIII can result in HTG with a highly significant linear relation between plasma apo CIII and plasma triglyceride concentrations (Fig. 4). Our results suggest that the apo CIII concentration may regulate the metabolism of triglyceride-rich lipoproteins. Indirect evidence from a number of sources is compatible with our observation. In vitro apo CIII can inhibit the lipolysis of triglyceride-rich lipoproteins by lipoprotein lipase (*21, 22*). In hypertriglyceridemic patients, a plasma inhibitor of lipoprotein lipase copurifies with apo CIII (*23*), and some studies have shown that hypertriglyceridemics have an elevated apo CIII/apo CII ratio (*24, 25*). Turnover studies in hypertriglyceridemic subjects indicate oversynthesis of apo CIII (*26*). Finally, fasting triglycerides were reduced in two sisters with genetic apo A-I and apo CIII deficiency, and hydrolysis of their VLDL by lipoprotein lipase in vitro was inhibited by exogenous apo CIII (*27, 28*).

Primary or secondary apo *CIII* overexpression may underlie many of the hypertriglyceridemic states in humans. A Sac I restriction fragment length polymorphism (RFLP) in the 3′ untranslated region of the apo *CIII* is associated with severe HTG in Caucasians (*29–31*). When considered together with our work, these results suggest that a common mutation may exist in linkage disequilibrium with this RFLP that can cause apo *CIII* overexpression and HTG, which could occur by transcriptional or posttranscriptional mechanisms. Studies on the transcriptional regulation of apo *CIII* have identified distal and proximal positive elements and a negative element that control liver-specific gene expression (*32*). Mutations in regulatory elements could lead to increased apo *CIII* expression. The apo *CIII* negative regulatory element is homologous to the interferon regulatory element (*32*). This element binds the transcription factor NF-κB, which mediates cytokine effects on gene expression (*33*). If NF-κB regulates expression of apo *CIII*, the HTG of infection or other altered physiological states might be explained by this mechanism.

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AIDS—The Leading Cause of Adult Death in the West African City of Abidjan, Ivory Coast

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In 1988 to 1989, 698 adult cadavers in Abidjan's two largest morgues were studied, representing 38 to 43% of all adult deaths in the city over the study period, and 6 to 7% of annual deaths. Forty-one percent of male and 32% of female cadavers were infected with human immunodeficiency virus (HIV). Fifteen percent of adult male and 13% of adult female annual deaths are due to acquired immunodeficiency syndrome (AIDS). In Abidjan, AIDS is the leading cause of death and years of potential life lost in adult men, followed by unintentional injuries and tuberculosis. In women, AIDS is the second leading cause of death and premature mortality, after deaths related to pregnancy and abortion. AIDS-specific and AIDS-proportional mortality rates may be higher in other African cities where AIDS has been found for a longer time than in Abidjan.

ALTHOUGH THE PREVALENCE OF INfection with HIV, the causative agent of AIDS, is high in many countries of sub-Saharan Africa (*1*), data concerning mortality due to AIDS are scarce. In Kinshasa, Zaire, the annual incidence of AIDS in adults was reported to be 500 to 1000 cases per million in 1985 (*2*), with an annual AIDS-specific adult mortality rate of at least 1 per 1000 (*1*). In Abidjan, Ivory Coast, where infection with both HIV-1 and HIV-2 occurs (*3*), the epidemic of AIDS has developed rapidly since recognition of the first AIDS cases in 1985. A recent study in this West African city documented a minimum annual incidence of AIDS of 1447 cases per million in adult men and 340 per million in adult women (*4*).

In African cities that have high rates of HIV infection, AIDS is having important, but little assessed, effects on patterns of